and/or increase the likelihood of radiation-induced toxicities. Prospective trials have shown that RTQA variations have a significant impact on the primary study end-point and could bias the analysis of the trial results[6]. A large prospective phase III (i.e. TROG 02.02) trial showed indisputably that poor radiotherapy resulted in suboptimal patient's outcomes. Moreover, the impact of poor quality radiotherapy delivery exceeded greatly the benefit of chemotherapy, thus biasing the primary end-point of this study. This large Australian trial provided a contemporary benchmark that future studies will need to exceed. Other specific consideration for RTQA in trials includes, but is not limited to, education of the accruing sites in RT-trial guidelines, promotion of consistency between centers and estimation of inter-patient and inter-institutional variations. Additionally, global cooperation is essential in the environment of common and rare cancers alike, in order to be able to create sufficiently large patient data sets within a reasonable recruitment period. This cooperation is not without issues and recently the need to have harmonized RTQA procedures has been strongly advocated by the Global Harmonisation Group. Ensuring RT compliance with protocol guidelines involves however gradually more resources-intensive procedures which are also labor intensive and are not cost-neutral. This will consequentially have a significant impact on the overall study budget. There are suggestion that QA programs are however cost-effective. This financial investment is of paramount importance, as non-adherence to protocol-specified RT requirements in prospective trials is very frequent. The European Organisation for the Research and Treatment of Cancer (EORTC) Radiation Oncology Group started to implement RTQA strategies in the 1980s, including on how to write a protocol for RT trials, defining RTQA procedures (such as benchmark case, dummy run and complex treatment dosimetry checks), assuring prospective individual case review feasibility and implementing an electronic data-exchange platform.

Keywords: Quality assurance, RTQA, prospective trial, patient's outcome, toxicity

SP-0233

What will we need for future RTQA in clinical trials?

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A trial protocol with clearly established delineation guidelines and dose-volume parameters is key to all RTQA. Acceptable and unacceptable variations thereof should be defined before the trial starts as these are the standards to which all RTQA data collected will be compared. The experience so far has been addressed by the previous two speakers. Dr. Miles presented the RTQA procedures in clinical trials, differentiating between pre-accrual and during accrual tasks. Thereafter, Dr. Weber clearly showed that non adherence to protocol-specified RT requirements is associated with reduced survival, local control and potentially increased toxicity. Thus, it can be concluded that clinical trial groups have established RTQA procedures and conformance to these procedures strengthen the trial results. In this talk the remaining issues that need to be solved will be addressed. These issues can be separated in:

1. How can we further optimising the current RTQA
2. How should we include new imaging and treatment modalities in our RTQA program?

The first part of the talk will address several initiatives to further optimise current RTQA procedures. As we have learned from past RTQA experience, currently the individual case reviews (ICRs) are the most common source of variations from trial protocols. ICR variation is also the most important RTQA factor affecting trial outcome. Thus, a transition is needed from retrospective ICRs to timely, full prospective ICRs. Also, with the further advancement of tailored treatments for small subgroups of patients there is a growing need for intergroup trials to increase the accrual rates when conducting trials for such patient groups. These changes place new requirements on multiple parts in the RTQA procedure:

- Standardisation of RTQA across various trial groups. The Global Harmonisation Group initiative.
- Standardisation of protocol requirements with clear definitions of acceptable and unacceptable variations.
- Standardisation of OAR and target naming conventions.
- Automated upload of RTQA data from institutions to the RTQA review organisation, including anonymisation software, use of Dicom standards.
- Metrics and software tools to automatically evaluate image quality, delineations and treatment plans.

The second part of the talk will address the ideas of including new diagnostic, treatment and evaluation modalities and techniques in RTQA programs. Examples will be shown of RTQA trial procedures for breathing correlated 4D-CT, 4D PET-CT, MRI and CBCT currently in use or under development.

Proffered Papers: Radiobiology 3: Novel targeting approaches in combination with radiation

OC-0234

Radiotherapy and L19-IL2: perfect match for an abscopal effect with long-lasting memory

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Purpose or Objective: There is conclusive evidence that radiotherapy (RT) can initiate an immune response. Previously, we have shown that addition of L19-IL2 to RT was able to increase the immune response and that this combination therapy resulted in a long-lasting synergistic anti-tumor effect. Here we hypothesize that tumor cells outside the radiation field will also be eliminated by this combination treatment (abscopal effect) and that tumors cannot be formed again after re-challenging cured animals (memory effect).

Material and Methods: Immunocompetent Balb/c mice were subcutaneously injected with syngeneic colorectal C51 cells in both flanks at different days. Primary tumors were irradiated upon a volume of 200 mm³ (15Gy or 5x2Gy) followed by PBS or L19-IL2 administration and the growth of the secondary non-irradiated tumors was monitored. Cured mice were reinjected after 150 days with C51 tumor cells and tumor uptake was assessed. Several immunological parameters in blood, tumors, lymph nodes and spleens were investigated in both experiments.

Results: RT+L19-IL2 was able to cure 100% of primary tumors and was associated with an increased percentage of CD8+ T cells inside these irradiated tumors. When a single RT dose of 15Gy was combined with L19-IL2, 20% of the non-irradiated secondary tumors were cured. Interestingly, the non-irradiated tumors of mice treated with 15Gy+L19-IL2 showed a significant ($p<0.01$) increased percentage of CD4+ T cells compared to irradiated tumors. Fractionated radiotherapy combined with L19-IL2 caused a significant ($p<0.01$) growth delay in the non-irradiated tumors, however no secondary tumors were cured. Immunological analysis revealed an increase in PD-1 expression on T cells infiltrating these tumors, suggesting a more regulatory phenotype after fractionated radiotherapy compared with one single RT dose. New C51 tumors were not able to form in cured mice whereas 100% of the age-matched control mice formed tumors that reached established end-points within 17 days. Splenic T cells of these cured mice were associated with a high expression of CD127.

Conclusion: Our data show that RT+L19-IL2 causes anti-tumor immune effects outside the radiation field and this effect is associated with an increase of CD4+ T cells. Cured mice are

not able to form new tumors and have high expression of CD127 on their T cells, a marker for immunological memory. This new treatment will be further investigated in a Phase I study for patients with an oligometastatic solid tumor (NCT02086721).

OC-0235

Enhancing stereotactic radiation schedules using the vascular disrupting agent OXi4503

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Purpose or Objective: The novel combretastatin analogue, OXi4503, is a vascular disrupting agent (VDA) that has recently been shown to significantly enhance a stereotactic radiation treatment. This was achieved using an OXi4503 dose of 10 mg/kg combined with a stereotactic treatment of 3 x 15 Gy. The current study was undertaken to determine the OXi4503 dose dependency when using different stereotactic radiation dose schedules.

Material and Methods: A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used in all experiments. Treatments were performed in restrained non-anaesthetised animals when tumours had reached 200 cubic mm in size. Tumours were locally irradiated (230 kV x-rays) with 3 fractions of radiation varying from 5-20 Gy (each fraction given with an interval of 2-3 days over a one week period). OXi4503 was dissolved in saline prior to each experiment; once prepared it was kept cold and protected from light. Various doses (5-25 mg/kg) were intraperitoneally injected into mice 1-hour after each irradiation treatment. Three days after the final irradiation the tumours were subjected to a clamped top-up dose which involved giving graded radiation doses with the tumour bearing leg clamped for 5 minutes before and during irradiation. The percentage of mice in each treatment group showing local tumour control 90 days after irradiating was then recorded. Following logit analysis of the clamped top-up radiation dose response curves, the TCD50 values (radiation dose to control 50% of tumours) were estimated. A Chi-squared test ($p<0.05$) was used to determine significant differences between the TCD50 values.

Results: The clamped top-up TCD50 values (with 95% confidence intervals) obtained following irradiation with 3 treatments of 10, 15 or 20 Gy were found to be 42 Gy (38-47), 30 Gy (23-39), and 0.8 Gy (0.3-2.3), respectively. A plot of the TCD50 values against the stereotactic doses gave rise to a linear response (slope = -4.1; correlation coefficient = 0.97). OXi4503 significantly decreased the clamped radiation top-up TDC50 values and this affect appeared to be independent of both the ambient radiation dose applied with each of the 3 fractions and the VDA dose; the curve showing the TCD50 values against stereotactic radiation dose was similar to that for radiation alone (slope = -4.3; correlation coefficient = 0.94), but the radiation + OXi4503 curve was some 15 Gy lower than the radiation only curve.

Conclusion: OXi4503 is an effective agent for enhancing a stereotactic radiation treatment. But, the enhanced response appeared to be a simple additive effect independent of both the radiation dose applied with each fraction and the VDA dose used.

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OC-0236

DTP-006: a novel, orally bioavailable hypoxia-activated prodrug

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Purpose or Objective: Hypoxia is a common feature of solid tumors. Conventional treatments such as chemo- and radiotherapy (RT) are less effective against hypoxic tumor cells. Hypoxia-activated prodrugs (HAPs) are specifically activated in hypoxia to target hypoxic cells as well as adjacent oxygenated tumor cells via their bystander effect. DTP-006 is a newly synthesized nitroaromatic HAP with highly favorable properties: 1) activation under hypoxia, 2) high bystander effect, 3) excellent aqueous solubility, 4) murine oral bioavailability and 5) no off-mechanism activation by human aerobic reductases NQO1 and AKR1C3. Here we show the effects of DTP-006 on tumor cell viability, spheroid growth and radiation resistant tumor cells *in vivo*, and assess its pharmacokinetics and oral bioavailability in mice.

Material and Methods: The one-electron reduction potential (E1) of DTP-006 was determined by pulse and steady state radiolysis. IC50 viability ratios were assessed in 2D cell culture exposed to normoxic or anoxic (2% O2) conditions. H460 multicellular layers (MCLs) under aerobic (5% CO2, 95% O2) or anoxic (5% CO2, 95% N2) conditions were incubated with DTP-006 for 5 h after which cells were plated for clonogenic survival. H460 spheroids were incubated with DTP-006 upon confirmation of a hypoxic core. NIH-III mice bearing H460 tumors received a single i.p. dose of DTP-006 (781 mg/kg) after irradiation (10 Gy) of tumors. 18 h later tumors were excised and single cell suspensions were generated and plated for clonogenic survival. Tumor-free female NIH-III mice received a single i.v. or oral dose of DTP-006 (383 mg/kg). Terminal blood samples collected at time points via cardiocentesis were analyzed by LC/MS/MS. Plasma half-life (T1/2) and absolute oral bioavailability (Fabs) were calculated.

Results: DTP-006 has an E1 value of -351 mV, indicating strong oxygen inhibition of nitro radical formation. IC50 were lower in anoxia than normoxia by factors of 203 (MDA-MB-468), 55 (C33A), and 20 (HCT116). In a H460 MCL clonogenic assay, 100 µM DTP-006 caused 99% cell kill under anoxia but exhibited no aerobic cell kill. It caused a concentration-dependent growth delay in spheroids, where 250 µM completely halted growth. A single dose of DTP-006 caused a significant loss of clonogenicity when combined with RT in an *in vivo* excision assay (log cell kill 2.35 relative to control). T1/2 after oral administration was 0.82 h and bioavailability was 47%.

Conclusion: DTP-006 kills tumor cells only in severe hypoxic conditions *in vitro*, reduces growth of tumor cell spheroids, and sterilizes radiation resistant tumor cells *in vivo*. It has clinically relevant bioavailability after oral administration. As such, DTP-006 is a promising new HAP with potentially favorable properties for clinical use. Further studies to determine the antitumor effects of DTP-006 as a monotherapy and in combination with RT in several preclinical tumor models are ongoing.

OC-0237

Adding Notch inhibition increases efficacy of standard of care treatment in glioblastoma

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